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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF CELECOXIB AND PARACETAMOL IN ITS BULK AND TABLET DOSAGE FORM

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Abstract:

The present study describes the development and validation of a simple, accurate, and precise Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Celecoxib and Paracetamol in bulk and pharmaceutical tablet dosage forms. Chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 × 150 mm, 5.0 µm) with a mobile phase of Methanol: Water (70:30 v/v) at a flow rate of 1 ml/min. Detection was performed at 260 nm, with an injection volume of 10 µl and a run time of 14 minutes under ambient temperature. Both drugs were well resolved with sharp, symmetrical peaks, and the method was validated in accordance with ICH guidelines. The results confirmed the method to be linear, accurate, precise, specific, robust, and sensitive. Recovery studies showed values close to 100%, indicating accuracy, while %RSD values within acceptable limits demonstrated reproducibility. The method successfully estimated the active pharmaceutical ingredients without interference from excipients. This validated RP-HPLC method can be effectively applied for the routine quality control and analysis of Celecoxib and Paracetamol in bulk drugs as well as in their combined tablet formulations.

Keywords: *RP-HPLC, Celecoxib, Paracetamol, Phenomenex Luna C18, simultaneous estimation, validation.*

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INTRODUCTION:

Pharmaceutical Analysis is used to determining the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more of these components.

For analyzing the drug samples in bulk, pharmaceutical formulations and biological fluids, different analytical methods are routinely being used. In non-instrumental, the conventional and physicochemical property are use to analyze the sample. The instrumental methods of analysis are based upon the measurements of some physical property of substance using instrument to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical methods. Therefore, analytical methods developed using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC have wide applications in assuring the quality and quantity of raw materials and finished products.

Analytical chemistry is the branch of chemistry focused on the identification and quantification of chemical substances. It involves the use of various techniques and instruments to analyze the composition of materials, determine their molecular structure, and understand their properties. Analytical chemistry plays a vital role in many fields, including pharmaceuticals, environmental science, forensics, food safety, and materials science.

The field is typically divided into two main types of analysis:

1. **Qualitative analysis** – Determines what substances are present in a sample.
2. **Quantitative analysis** – Measures how much of a specific substance is present.

Key techniques in analytical chemistry include:

- Spectroscopy (e.g., UV-Vis, IR, NMR)
- Chromatography (e.g., HPLC, GC)
- Mass spectrometry
- Titration
- Electrochemical analysis

1. PRINCIPLE OF CHROMATOGRAPHY:

Adsorption Chromatography:

When the stationary phase is a solid and mobile phase is liquid or gaseous phase, it is called Adsorption Chromatography.

Examples: Thin layer chromatography,

Partition Chromatography:

When the stationary phase and mobile phase are liquid, it is called Partition Chromatography.

Example: Paper partition chromatography, Gas-liquid.

Phases of Chromatography

Normal Phase Chromatography:

In Normal Phase mode the stationary phase is polar and the mobile phase is non-polar in nature. In this technique, non-polar compounds travel faster and are eluted first. This is because of the lower affinity between the non-polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

Reversed Phase Chromatography:

It is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode the stationary phase is non polar hydrophobic packing with octal or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. The polar compound gets eluted first in this mode and non-polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are Octa Decyl Silane (ODS) or C18, C8, C4, (in the order of increasing polarity of the stationary phase). An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity

MATERIALS AND METHODS:

INSTRUMENTS USED

HPLC from WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.

CHEMICALS USED:

Celecoxib and Paracetamol from Sura Pharma Labs, Water and Methanol for HPLC from LICHROSOLV (MERCK).

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Celecoxib and Paracetamol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2.25ml of the above Celecoxib and 0.45ml of the Paracetamol stock solutions into a

10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and water in proportion 70:30 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:
Accurately measured 700ml (70%) of HPLC Methanol and 300ml of Water (30%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Water (70:30v/v)
Column : Phenomenex Luna C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 260 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 14 minutes

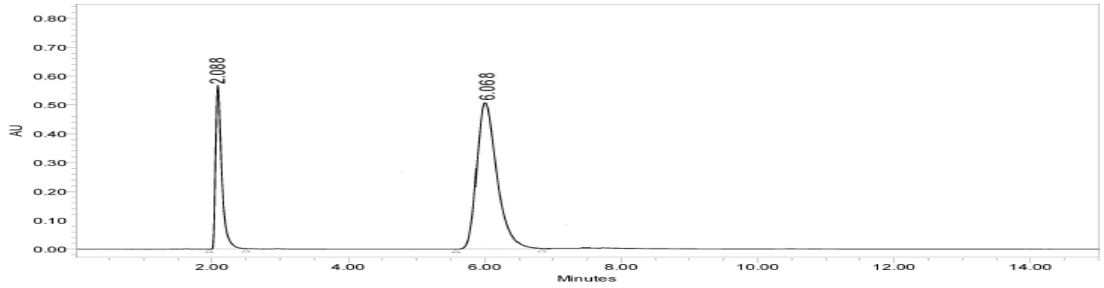

Fig-7.7: Optimized Chromatogram

Table7.7: Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Celecoxib	2.088	3425413	567933		1.0	5565.5
2	Paracetamol	6.068	1629854	517733	2.5	1.1	5355.2

Observation:
From the above chromatogram it was observed that the Celecoxib and Paracetamolpeaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

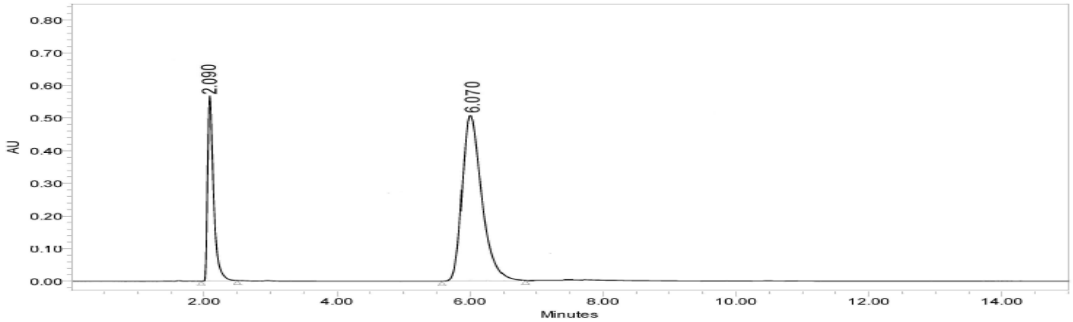

Figure-7.8: Optimized Chromatogram (Sample)

Table-7.8: Optimized Chromatogram (Sample)

S.No.	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Celecoxib	2.090	3468547	567933		1.0	5565.5
2	Paracetamol	6.070	16289441	517733	2.5	1.1	5355.2

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank:

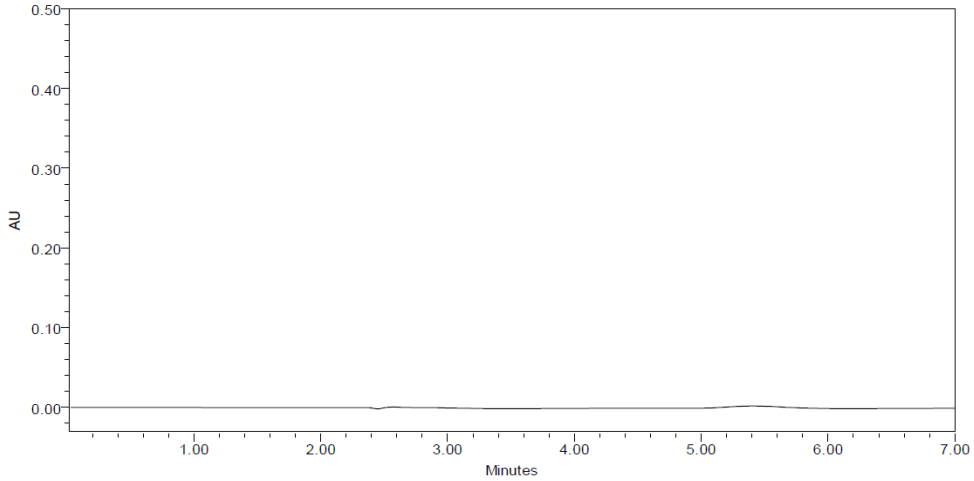

Fig-7.9: Chromatogram showing blank (mobile phase preparation)

System Suitability:

Table-7.9: Results of system suitability for Celecoxib

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Celecoxib	2.080	3569412	567917	5568.0	1.0
2	Celecoxib	2.080	3465125	517719	6359.2	1.1
3	Celecoxib	2.080	3598154	567933	5565.5	1.0
4	Celecoxib	2.081	3586491	517733	5355.2	1.1
5	Celecoxib	2.081	3582694	567917	6348.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table-7.10: Results of method precession for Paracetamol:

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Paracetamol	2.080	3582264	567917	5568.0	1.0	2.5
2	Paracetamol	2.080	3586491	517719	5359.2	1.1	2.5
3	Paracetamol	2.080	3598154	567933	5565.5	1.0	2.5
4	Paracetamol	2.081	3564125	517733	5355.2	1.1	2.5
5	Paracetamol	2.081	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Celecoxib and Paracetamol in drug product.

Assay (Standard):

Table-7.11: Peak results for assay standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Celecoxib	2.087	3425681	567917		1.0	5568.0	1
2	Paracetamol	6.067	1623598	517719	2.5	1.1	5359.2	1
3	Celecoxib	2.088	3425413	567933		1.0	5565.5	2
4	Paracetamol	6.068	1629854	517733	2.5	1.1	5355.2	2
5	Celecoxib	2.088	3425413	567933		1.0	5545.5	3
6	Paracetamol	6.068	1629854	517733	2.5	1.1	5352.1	3

Assay (Sample):

Table-7.12: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Celecoxib	2.089	3469821	567917		1.0	6568.0	1
2	Paracetamol	6.069	16259845	517719	2.5	1.1	5359.2	1
3	Celecoxib	2.090	3468547	567933		1.0	5565.5	2
4	Paracetamol	6.070	16287531	517733	2.5	1.1	5355.2	2
5	Celecoxib	2.090	3468143	567813		1.0	5391.1	3
6	Paracetamol	6.070	16282431	517623	2.5	1.1	5564.0	3

Table7.13: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Celecoxib	100mg	20	100.1%
2	Paracetamol	50mg	10	100.5%

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Celecoxib and Paracetamol in pharmaceutical dosage form was found to be 100.1%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Celecoxib:

Concentration µg/ml	Average Peak Area
5	1010252
10	2049374
15	3072706
20	3921068
25	4952813

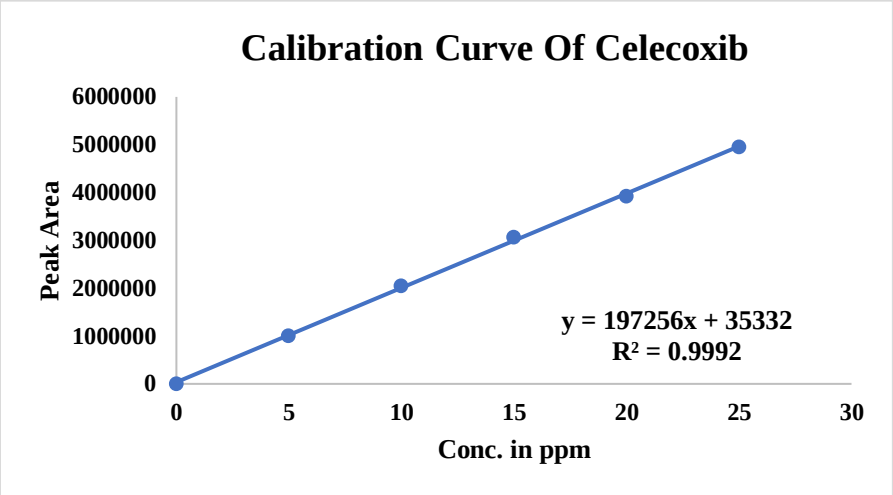

Figure7.26: Calibration Graph for Celecoxib

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Celecoxib is a straight line.

Y = mx + c

Slope (m) = 197256

Intercept (c) = 35332

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 35332. These values meet the validation criteria.

Paracetamol

Concentration µg/ml	Average Peak Area
0	0
15	8040807
30	14318417
45	21087985
60	27913928
75	34584741

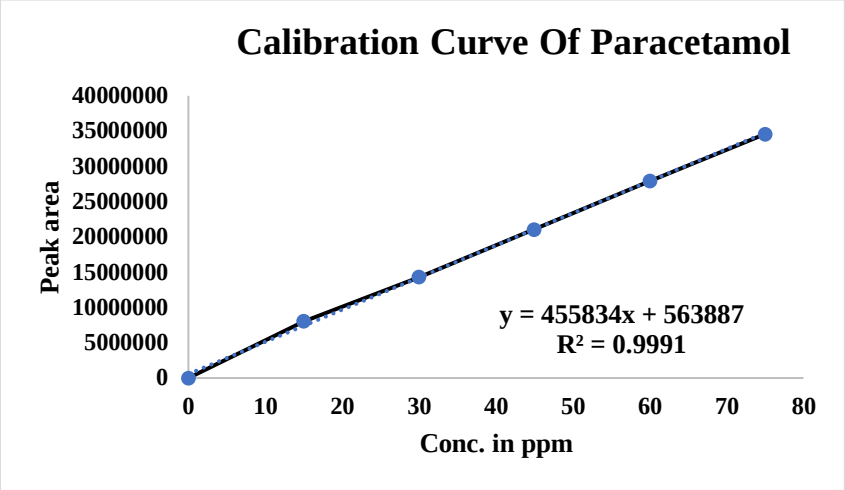

Figure 7.27: Calibration Graph for Paracetamol

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Paracetamol is a straight line.

Y = mx + c

Slope (m) = 455834

Intercept (c) = 563887

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 56388. These values meet the validation criteria.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-7.14: Results of repeatability for Celecoxib

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Celecoxib	2.084	3569412	567917	5568.0	1.0
2	Celecoxib	2.083	3465125	517719	5359.2	1.1
3	Celecoxib	2.082	3598154	567933	5565.5	1.0
4	Celecoxib	2.081	3586491	517733	5355.2	1.1
5	Celecoxib	2.080	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-7.15: Results of method precision for Paracetamol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Paracetamol	2.080	3582264	567917	5568.0	1.0	2.5
2	Paracetamol	2.081	3586491	517719	5359.2	1.1	2.5
3	Paracetamol	2.082	3598154	567933	5565.5	1.0	2.5
4	Paracetamol	2.083	3564125	517733	5355.2	1.1	2.5
5	Paracetamol	2.084	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Day 1:

Table-7.16: Results of Intermediate precision for Celecoxib

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Celecoxib	2.081	3481579	567917	5568.0	1.0
2	Celecoxib	2.082	3458121	517719	5359.2	1.1
3	Celecoxib	2.083	3426581	567933	5565.5	1.0
4	Celecoxib	2.084	3465712	517733	5355.2	1.1
5	Celecoxib	2.085	3451476	567917	5568.0	1.0
6	Celecoxib	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.

Table-7.17: Results of Intermediate precision for Paracetamol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Paracetamol	6.061	15481579	567917	5568.0	1.0	2.5
2	Paracetamol	6.062	15369852	517719	5359.2	1.1	2.5
3	Paracetamol	6.063	15248454	567933	5565.5	1.0	2.5
4	Paracetamol	6.064	15874692	517733	5355.2	1.1	2.5
5	Paracetamol	6.064	15236547	567933	5568.0	1.0	2.5
6	Paracetamol	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table-7.18: Results of Intermediate precision Day 2 for Celecoxib

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Celecoxib	2.081	3481579	567917	5568.0	1.0
2	Celecoxib	2.082	3458121	517719	5359.2	1.1
3	Celecoxib	2.083	3426581	567933	5565.5	1.0
4	Celecoxib	2.084	3465712	517733	5355.2	1.1
5	Celecoxib	2.085	3451476	567917	5568.0	1.0
6	Celecoxib	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.

Table-7.19: Results of Intermediate precision for Paracetamol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Paracetamol	6.061	15481579	567917	5568.0	1.0	2.5
2	Paracetamol	6.062	15369852	517719	5359.2	1.1	2.5
3	Paracetamol	6.063	15248454	567933	5565.5	1.0	2.5
4	Paracetamol	6.064	15874692	517733	5355.2	1.1	2.5
5	Paracetamol	6.064	15236547	567933	5568.0	1.0	2.5
6	Paracetamol	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Table-7.23: The accuracy results for Celecoxib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	15	15.2	101.9	100.9%
100%	3035883	30	30.4	101.4	
150%	4451005	45	44.7	99.4	

Table-7.24: The accuracy results for Paracetamol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	30	30.07	100.2	99.6%
100%	2096069	60	59.6	99.4	
150%	3112684	90	89.3	99.3	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table-7.25: Results for Robustness

Celecoxib :

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Paracetamol:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

8. SUMMARY AND CONCLUSION:

A simple, precise, and reproducible Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Celecoxib and Paracetamol in bulk and pharmaceutical tablet dosage form. The chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm) with a mobile phase of Methanol:Water (70:30 v/v), delivered at a flow rate of 1 ml/min. The detection was carried out at 260 nm with an injection volume of 10 µl under ambient temperature. The optimized method provided a satisfactory run time of 14 minutes with well-resolved, sharp, and symmetric peaks for both analytes. The method was validated as per ICH guidelines and found to be linear over the studied concentration range for both drugs. Accuracy, precision, robustness, specificity, LOD, and LOQ results confirmed the reliability of the developed method. The recovery studies demonstrated good accuracy with recoveries close to 100%, and the precision study indicated reproducibility with low %RSD values.

CONCLUSION

The developed RP-HPLC method proved to be accurate, sensitive, specific, and robust for the simultaneous estimation of Celecoxib and Paracetamol in both bulk and tablet dosage forms. The optimized chromatographic conditions—mobile phase Methanol:Water (70:30 v/v), Phenomenex Luna C18 column, flow rate 1 ml/min, detection at 260 nm, and run time of 14 minutes—ensured efficient separation and quantification of the drugs without interference from excipients.

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